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# Investigations Into the Cross-Infectivity of Nematode Parasites of Cattle and Sheep

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Investigations Into the Cross-Infectivity  
of Nematode Parasites of Cattle and Sheep

A thesis submitted in partial fulfillment  
of the requirements for the degree of  
Master of Science in Animal Science

by

Eva Wray  
University of Arkansas  
Bachelor of Science in Animal Science, 2011

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This thesis is approved for recommendation to the Graduate Council.

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## Abstract

The existence of anthelmintic resistance is not singular in parasite species, host species or geographic region. It has become a problem of worldly stature, especially when considering sheep nematodes. Recommendations have been modified from chemical-based control to multipart, managerial intervention. Compared with nematodes of cattle, the worldwide resistance of sheep nematodes to anthelmintic remedies is much more pronounced and urgent. As the resistance to essentially all anthelmintic classes approaches a cautionary level, the implementation of non-chemical controls has grown increasingly essential. Six-6 to 8 month old Holstein steer calves, six-5 month old Katahdin ram lambs and eight-5 month old Suffolk X Rambouillet lambs were dewormed and inoculated with either cattle-source infective nematode larvae or sheep-source infective nematode larvae. Fecal egg counts (FEC) were followed until necropsy at 39 and 40 days post inoculation. The total mean FEC were highest amongst the hair and wool sheep groups, respectively, which were inoculated with both sheep-source and cattle-source nematodes. The low fecal egg output by treatment group 1 shows that using cattle as models for the sheep-source nematodes can keep the fecundity of the nematodes low; however, caution should be taken before implementation. The host specificity of *Haemonchus* spp. seems to be a minimal factor in nematode fecundity.

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## 1. Introduction

The existence of anthelmintic resistance is not singular in parasite species, host species or geographic region. It has become a problem of worldly stature, especially when considering sheep nematodes. Recommendations have been modified from chemical-based control to multipart, managerial intervention. Producers are now encouraged to not rely exclusively on chemicals to control internal parasites, but to incorporate additional novel methods to aid in the control. This research concentrates on the possibility of using alternate species rotation as a novel means of parasite regulation.

### *Typical Trichostrongyle life cycle*

The nematode egg in an early zygote stage is excreted from the animal in the feces. If the environmental conditions, including ambient temperature, moisture content and oxygen availability, are ideal, embryonation of the egg occurs immediately (Yazwinski and Tucker, 2006b). After approximately two days, a first stage larva (L<sub>1</sub>) emerges from the egg. The L<sub>1</sub> sheds its protective sheath and feeds on the bacteria in the fecal pat. After approximately two days, the L<sub>1</sub> enters lethargy and undergoes ecdysis to become a second stage larva (L<sub>2</sub>). The L<sub>2</sub> sheds its protective sheath and feeds on the bacteria and organic matter in the fecal pat. After approximately three days, the L<sub>2</sub> enters lethargy and undergoes ecdysis to become a third stage, infective larva (L<sub>3</sub>). The L<sub>3</sub> exsheaths, but does not shed, its protective sheath. This retained sheath acts as protection as the L<sub>3</sub> migrates away from the fecal pat onto the pasture. Most trichostrongyle L<sub>3</sub> are negatively geotropic and migrate up the grass blades when wet. The L<sub>3</sub> is ingested by a suitable, grazing host animal; this, initiating the start of prepatency. The L<sub>3</sub> sheds its protective sheath within hours of being ingested. Ecdysis of L<sub>3</sub> into parasitic fourth stage

larvae (L<sub>4</sub>) usually occurs in or on the mucosal lining approximately 7 days post infection. This stage in the nematode life cycle acts as a command stage, as the L<sub>4</sub> moves itself to the location inside the host animal where maturation can continue and adjustments are made to maintain optimal conditions for overall burden survival. The fourth (final) ecdysis occurs when the L<sub>4</sub> molts into a parasitic fifth stage larva, or immature, early adult; a transition that occurs approximately 20 days post infection. The immature, early adults mature into reproductive (dioecious) adults approximately in 15 to 40 days post infection. Eggs are produced and patency begins. Natural adult nematode death occurs in 1 to 10 months, depending on the nematode species and overall physiological status of the host (Yazwinski and Tucker, 2006b).

Typical physical features displayed by nematodes of ruminants include: (1) an elongated, cylindrical body; (2) a mouth and an anus/cloaca located at opposite ends of the body; (3) extreme sexual dimorphism; and, (4) possession of a filariform esophagus. The nematodes also are typically monoxenous, dioecious, reproductive only at the adult stage and oviparous.

*Specifics of the major ruminant genera:*

*Cooperia* spp (Ransom, 1907)

*Cooperia* spp display the normal trichostrongyle appearance and display the typical trichostrongyle life cycle. Mature adult cooperiads possess a cuticle that is striated and distended at the anterior end, giving the head a distended or rounded appearance. The cuticle has 14-16 longitudinal lines, which are striated. Mature females have straight tails and a non-descript, longitudinal vulva with cuticular ridges. Female “bending” at the vulva is characteristic at the level of species. Mature male cooperiads possess a bursa that is comprised of two lateral lobes

and a small dorsal lobe. The spicules are medium-sized, robust, pointed at their distal ends and with a wing-like expansion located in the middle. No gubernaculum is present. *C. oncophora* (Figure 3e.) are bigger than *C. punctata* (Figure 3d.); 5.5-9 mm versus 4.7-5.9 mm. Prepatency and patency for this nematode are 11-22 days and 1-9 months, respectively.

*Cooperia* resides in the proximal small intestine of cattle. Neither histophagic nor “heavily” hematophagic, this nematode is a “true grazer”. Though there is no tissue invasion, small intestine villi become constricted and atrophied. Significant burdens are limited to animals under two years of age and can cause significant pathology (Yazwinski et al., 2013b).

Pathogenicity of cooperiasis is usually mild and includes (1) lowered feed efficiency, (2) intestinal inflammation, (3) diarrhea and (4) subsequent dehydration. Cooperiasis is restricted to cattle, with small ruminants spared this genus except for *C. curticei*, which is rare in the USA.

*Haemonchus* spp (Cobb, 1898)

*Haemonchus* spp have typical nematode physical features and are voracious, hematophagic nematodes located in the abomasum of ruminants. Mature, adult *Haemonchus* can be identified by their large size. Mature females have a pointed tail and a blood-filled intestine which is spiraled around a white ovary, giving rise to the moniker, “barber pole worm”. The vulva of the female is behind the middle of the body and is covered by a vulvar process. Mature males have a long and prominent bursated tail with hefty lateral lobes and a petite irregular dorsal lobe. The hooked spicules are long, robust, pointed and a gubernaculum is present. Both species of *Haemonchus* (*H. contortus* (Figure 3b.) and *H. placei* (Figure 3a.)) are similar in size, with



males being 10-20 mm in length and females being 18-30 mm in length. *H. placei* and *H. contortus* are primarily cattle and small ruminant nematodes, respectively.

In the absence of interruptions, *H. placei* develops in the typical trichostrongyle manner and can live as a mature adult for as long as 8 months. *H. contortus* exhibits a winter inhibition within the abomasal mucosa. Uninterrupted prepatency is 18-21 days and larval development is dependent on season and host reproductive status; active and pathogenic during the spring and summer months, while inhibited and arrested during the winter months. *Haemonchus* is a highly fecund nematode, with egg production reaching 5,000 eggs per mature adult female per day. (Kaplan, 2010) *Haemonchus* exhibits intraburden inhibition in response to host animal blood loss. Extra-host pasture stages of *Haemonchus* spp are resistant to hot/dry conditions as they exhibit anhydrosis.

Pathogenesis of haemonchosis includes anemia, submandibular edema, diarrhea, weakness, and rapid weight loss. (Kaplan, 2006) Clinical signs include ‘bottle jaw’ and hydrothorax. In hyperacute infections, significant blood loss can lead to sudden death (Yazwinski, unpublished). Pathology at the abomasal level includes mucosal swelling, petechial hemorrhages and shallow ulcers, mostly occurring during the prepatent period.

*Oesophagostomum* spp (Molin, 1861)

*Oesophagostomum* spp are robust nematodes and display the normal trichostrongyle life cycle, with an additional unique feature of transcutaneous infection, followed by tracheal migration (Yazwinski et al., 2013a). This nematode is a relatively large worm with a short buccal capsule. The mouth is surrounded by a cuticular mouth band (mouth band is thicker and more defined in *O. radiatum* [cattle species] (Figure 3f.) when compared to *O. venulosum* [sheep species] (Figure 3g.). Mature females have pointed tails and they often bear a copulatory plug. Mature males possess a small bursate tail with long spicules and a gubernaculum. The two most dominant species are relatively similar in size; 14-17 mm and 16-22 mm (*O. radiatum* male and female, respectively) versus 11-16 mm and 13-24 mm (*O. venulosum* male and female, respectively).

*Oesophagostomum* spp has a long prepatent period of 45-60 days and has segmented populations at both the L<sub>4</sub> and adult stages, with the fourth stage infection occurring primarily in the small intestine and the adult infection occurring in the proximal large intestine of ruminants. The parasitic period for this nematode can reach 15 months. *Oesophagostomum* L<sub>4</sub>s cause a histotrophic response by the host animal in the terminal small intestine submucosa after sensitization, giving rise to the common worm name “Nodular Worm”. Nodules are formed primarily during the challenge infection. The L<sub>4</sub> will either die or break out of the nodules to continue with the life cycle. Nodule formation and L<sub>4</sub> emergence account for the majority of the pathology associated with this nematode. (Goldberg, 1951)

Pathogenesis of oesophagostomiasis is primarily due to the larval “nodulation” and emergence. The L<sub>4</sub> induce inflammation, but can also cause abscesses about 2 mm in diameter. The nodules are larger in size and the immune response is much more extensive during challenge infections.

Aside from the physical damage caused by *Oesophagostomum* spp L<sub>4</sub>, severe diarrhea, anorexia, dehydration and emaciation may occur.

*Ostertagia ostertagi* (Ransom, 1907)

*Ostertagia ostertagi* (Figure 3c.) possesses a typical trichostrongyle appearance and follows the typical trichostrongyle life cycle. *O. ostertagi* are medium sized worms with a straight head and a small buccal capsule. The cuticle bears 25-30 longitudinal indentations. Mature females possess” pointed tails and a vulva that opens in a slanting slit that is covered by a cuticular flap, though “flapless” females occasionally occur. Mature males possess a short bursated tail with two large lateral lobes and a small dorsal lobe that is usually bent at the anterior portion. The spicules are small and equal in size, ending with three bluntly hooked processes. A gubernaculum is present. Mature adults are 6.7-7.5 mm and 8.3-9.2 mm in length (male and female, respectively). Prepatency and patency for *O. ostertagi* is 16-23 days and 2-3 months, respectively. Being reddish brown in color as adults, this worm has been denoted the “Brown Stomach Worm”.

*Ostertagia* resides in the abomasum of cattle and is a “grazer” in nature. This nematode displays seasonal inhibition, resulting in Type I, Pre-Type II and Type II ostertagiasis. Type I is the uninterrupted life cycle with the infection comprised primarily of adults. Pre-Type II is the arrestment of early fourth stage larvae (IEL<sub>4</sub>) in the abomasal wall. Type II is the activation of the IEL<sub>4</sub> and the final progression of the life cycle. In the southern United States, Type I ostertagiasis occurs during the winter months (fall, winter and early spring). Pre-Type II ostertagiasis occurs during late spring and early summer and Type II ostertagiasis occurs in late

summer. (Williams et al., 1987) In the northern United States, the arrestment (pre-type II) occurs during the winter months. Type II is the most pathogenic form of ostertagiasis, as the massive emergence tends to overwhelm the host. (Edmonds et al., 2013) *O. ostertagi* “counterpart” in small ruminants is *Teladorsagia circumcincta*.

Pathogenesis of ostertagiasis/teladorsagiasis includes abomasitis, edema, usurped effective abomasal mucosa due to nodules formed by IEL<sub>4</sub>s and abomasal “leakage” resulting in hypoproteinaemia due to disruption of the abomasal wall by the IEL<sub>4</sub>s. (Williams et al., 1987) The abomasal mucosa destruction results from small prominent nodules (“Moroccan Leather”) or inflamed hemorrhagic areas about 1-2 mm in diameter. “All things considered”, *O. ostertagi* is probably the most important nematode of cattle in the U.S. (Yazwinski et al., 2013b).

*Trichostrongylus colubriformis* (Giles, 1892)

*Trichostrongylus colubriformis* has a typical nematode appearance and displays a normal trichostrongyle life cycle. Mature adult *Trichostrongylus* are small nematodes with an excretory pore on an otherwise nondescript, straight head. Mature females have straight tails with a vulva stationed at the middle of the body that opens in a longitudinal slit without protruding edges. Ovijectors are well visible. Mature males have bursated tails with large lateral lobes and a well-defined, balanced dorsal lobe. The spicules are brownish, unequal and bent ventrally and a gubernaculum is present (Figure 3i.). Adult males are 4.3-7.7 mm in length and adult females are 5.0-8.6 mm in length. Prepatency and patency for this nematode is 15-23 days and 12-70 days, respectively.

*T. colubriformis* resides in the proximal small intestine in sheep, does not display any larval arrestments and has relatively low pathogenicity. *T. colubriformis* displays low fecundity and produces <200 egg per day. Pathogenesis of trichostrongyliasis includes mucosal irritation and inflammation with subsequent swelling and edema. *T. colubriformis* is the second most abundant nematode of small ruminants in the USA, falling far behind *H. contortus* in importance and magnitude.

Miscellaneous nematodes:

*Chabertia ovina* (Railliet and Henry, 1909)

*Chabertia ovina* (Figure 3j.), denoted the “large-mouth bowel worm” of ruminant animals (primarily sheep), displays a normal trichostrongyle appearance and exhibits a normal trichostrongyle life cycle. Considered more important in Australia than in the U.S., *C. ovina* are large nematodes, with mature males measuring 13-14 mm long and mature females reaching 17-20 mm in length. The distinguishing physical attribute of this worm is its “bowl-shaped” buccal capsule. Prepatency for *C. ovina* is very long (upwards of 11 weeks). The larval stages are blood suckers and cause a histotropic response by the host animal; these conditions can persist for 1-2 months.

*Teladorsagia circumcincta* (Ransom, 1907)

*Teladorsagia circumcincta* displays a normal trichostrongyle appearance and exhibits a normal trichostrongyle life cycle. *T. circumcincta* is the most important sheep nematode in Britain.

Mature males measure 7.5-8.5 mm long and possess slender, trifurcated spicules and a

gubernaculum. Fecund females are 9.8-12.2 mm in length. The cattle “counterpart” of this nematode is *Ostertagia spp.*

### *Aspects of the chemical control of nematodes*

#### *The current anthelmintic classes:*

##### 1. *Macrocyclic Lactones*

Macrocyclic lactones comprise a class of anthelmintic that is composed of two groups: avermectins (e.g. ivermectin, doramectin, abamectin, selamectin, eprinomectin) and milbemycins (e.g. moxidectin). Both groups have primary activity targeting glutamate-gated ion exchange gates in the cellular membrane of parasitic nerves and muscles. These chemicals bind the ion channels, allowing for the unaltered influx of chloride ions. (Wolstenhome and Martin, 2014) Binding to the glutamate-gated chloride (GluCl) channel receptors in many locations accounts for the variable paralytic effects on different neuromuscular systems; (pharynx, body wall and uterine muscles of nematodes).

An important aspect of macrocyclic lactones is the fact that they are hydrophobic in nature and accumulate in the adipose tissue, regardless of administration route. The lipophilicity of macrocyclic lactones differs among the chemical types (*ivermectin*<*doramectin*<*eprinomectin*<*moxidectin*) with moxidectin being nearly 100 fold more lipophilic than ivermectin. Macrocyclic lactones are primarily excreted in the feces and urine, but the more lipophilic compounds also may be excreted in the milk. In addition to lipophilicity and attachment-affinity at the GluCl gates, the macrocyclic

lactones differ greatly in other specificity to multidrug resistant (MDR) receptors possessed by the target parasites; a major factor in macrocyclic lactone effectiveness and nematode resistance to the chemical class.

## 2. *Benzimidazoles*

Benzimidazoles comprise a class of anthelmintic that include the “White Dewormers” (e.g. fenbendazole, albendazole, oxfendazole). These anthelmintics cause death of the nematode by depolymerization of microtubules. It is assumed that the inhibition of energy metabolism is a “downstream” effect of depolymerization of  $\beta$ -tubulin and this plays an essential role in benzimidazole lethal activity toward nematodes. Resistance to one drug in this class of anthelmintics will result in a cross-resistance to all other members of the same class.

## 3. *Imidazothiazole/Tetrahydropyrimidine*

This class of anthelmintics includes levamisole and tetrahydropyrimidines. These two chemical classes mimic the effects of acetylcholine; depolarizing efferent nerves resulting in spastic paralysis. Newby et al., 1985) Imidazothiazoles and tetrahydropyrimidines act as agonists at the nicotinic acetylcholine receptors (nAChRs) of nematodes, with two phases of action via stimulation of somatic muscle cells: hypercontraction of body wall muscles followed by a complete paralysis.

### *Anthelmintic-resistance in cattle nematodes*

Anthelmintic resistance has seemingly become a worldwide problem amongst livestock nematodes, and though there is still a benefit in utilizing chemicals in parasite control regimens, some classes have become non-efficacious. (Stromberg and Gasbarre, 2006; Jackson et al., 2006; Loveridge et al., 2003) The introduction and subsequent explosion of use of the macrocyclic lactones in the mid-70s has led to a rising, substantial resistance in nematodes. Denoted the “wonder drug”, this chemical class has generated various reviews of efficacy. (Geary, 2005; Gasbarre et al., 2009; Loveridge et al., 2003; Anziani et al., 2004; Loveridge et al., 2003; Fiel et al., 2001; Yazwinski et al., 2009b; Kaplan and Vidyashankar, 2012) Milbemycin is still displaying high levels of efficacy. (Cleale et al., 2004; Yazwinski et al., 2006b; Yazwinski et al., 2013a; Ives et al., 2007) Conflicting reports have been published for imidazothiazoles: Lyndal-Murphy et al. found that the chemical class is still efficacious, but Becerra-Nava et al. reported that it is losing its effectiveness. Benzimidazoles resistance does not appear to be an issue with cattle, but it is rampant in small ruminant and horses.

#### *Anthelmintic-resistance in sheep nematodes*

Compared with nematodes of cattle, the worldwide resistance of sheep nematodes to anthelmintic remedies is much more pronounced and urgent. (Cezar et al., 2010; Mitchell et al., 2010) Though all ovine nematodes have displayed resistance, the main concern in the development of resistance is *H. contortus*, which has displayed resistance to all classes of drugs that are currently available: avermectins (Cezar et al., 2010; Kaplan and Vidyashankar, 2012; Howell et al., 2008; Love et al., 2003), milbemycins (Cezar et al., 2010; Kaplan and Vidyashankar, 2012; Howell et al., 2008;), benzimidazoles (Cezar et al., 2010; Kaplan and Vidyashankar, 2012; Mitchell et al., 2010; Howell et al., 2008; Barrere et al., 2013 ) and imidazothiazoles (Cezar et al., 2010; Mitchell et al., 2010). Special attention should be given to



this “genetically superior” nematode if there is to be a profitable future for the small ruminant industry.

#### *In vivo anthelmintic evaluation methods*

More often than not, internal parasite infections go unnoticed by the producer, as the animal can be asymptomatic. This creates a problem in the farm-to-farm evaluation of parasite management. One method of resistance evaluation is via the fecal egg count reduction test (FECRT). A set of FECRT “rules” are laid out in Yazwinski’s 2013 paper, “Considerations for control of helminths in stocker cattle.” This *in vivo* efficacy evaluation has been used by many researchers in recent years. (Gasbarre, 2009; Jackson et al., 2006; Yazwinski et al., 2009a; Yazwinski et al., 2009b; Barrere et al., 2013; Condi et al., 2009; Rocha et al., 2008)

Another, more accurate, *in vivo* method of efficacy evaluation is the control study. This involves the comparison of treatment groups to a non-treated group, with both groups being sacrificed at the termination of the study and the internal nematode burdens compared. (Yazwinski et al., 2013b) Though a control study is a conclusive assessment of efficacy, it is difficult and very expensive to carry out. (Yazwinski et al., 2013b)

#### *Refugia*

*Refugia* is defined as the population of nematodes present on a given farm that has not been exposed to a given treatment. The larger the unexposed population of nematodes, the lower the selection for the development of resistance. (Van Wyk, 2001) Though *refugia* has been a well-known concept in disease control for many decades, it has long been overlooked in regards to an integral part of the solution to anthelmintic-resistant nematodes. In 2001, Van Wyk stated, “Farmers should be educated to consider *refugia* above all else when designing worm

*management programmes.*” Many other parasitologists have reiterated the importance of *refugia* when implementing a parasite management program. (Besier, 2012; Gasbarre, 2014)

### *“Smart Drenching”*

An emerging management recommendation is the concept of “smart drenching”. (Kaplan, 2006; Kaplan, 2010) This encompasses many aspects of deworming practices being properly combined and implemented in order to reduce the incidence of resistance on a given operation. These considerations include proper dosing, rotation of anthelmintic classes, combining anthelmintics of different modes of action, implementation of biosecurity, etc.

The implementation of proper dosing practices is extremely important in the reduction of resistance amongst nematode populations. Proper dosing encompasses administering correct doses to animals, administration of anthelmintics at the proper times, evaluating treatment effectiveness, etc. Implementation of FAMACHA scoring into your small ruminant deworming regimen can be very beneficial in slowing the reflection for anthelmintic resistance, as this system of anemia detection can help producers determine proper individual treatment and timing anthelmintic interaction to lead to a more conservative use of anthelmintic (Glaji et al., 2014; Nabukenya et al., 2014; Kaplan et al., 2004; Miller, 2011; Kaplan, 2006; Kaplan, 2010; Gasbarre, 2014)

The concepts of anthelmintic rotation and using anthelmintics in combination have been considered with differing conclusions. Anthelmintic rotation lacks the evidence that acting in such a manner will reduce the eventual incidence of resistance. Using anthelmintics in combination seemingly slows the propagation of resistance, as increased efficacy allows for fewer resistant nematode eggs to reach pasture. (Kaplan, 2006; Kaplan, 2010) These eventual

eggs, however, result in multi-drug resistant nematodes, as opposed to single-drug resistant worms.

Oversight practices can greatly impact the resistance status on a given operation. The concept of *oversight* entails not only the monitoring of nematode genetics on a given operation, but also performing the FECRT to determine anthelmintic resistance status. (Kaplan, 2006; Kaplan, 2010) Though the “treat and move” model has been a common practice amongst producers for decades, this dated methodology can actually drive resistance on operations to an unmanageable level. The practice entails treating an entire herd or flock (i.e. *blanket treating*), followed by moving the herd or flock to a relatively “clean” pasture. Doing so essentially distributes only resistant nematodes, and thus canceling the refugia on a pasture. (Kaplan, 2006; Kaplan, 2010)

#### *Aspects of non-chemical controls of nematodes*

As the resistance to essentially all anthelmintic classes approaches a cautionary level, the implementation of non-chemical controls has grown increasingly essential (small ruminants).

Anthelmintics cannot be relied on to control worms. Producers must employ different management strategies to combat anthelmintic resistance. These strategies include culling, alternate species grazing, nutritional supplementation and bioactive nutraceutical forages.

Grazing management is a tool for parasite control and has seemingly stood the test of the ever-changing alternative control methods. The grazing of two different species of animals, (species rotation, rotational grazing, co-species grazing, etc.) has been a recommended practice in order to help producers manage internal parasites. (Yazwinski and Tucker, 2006) The action of an alternate host animal ingesting infective parasites for which they are not susceptible can potentially lead to alteration of the nematode life cycle and most likely death of the parasite.

(Smith and Archibald, 1965; Rocha et al., 2008; Borgsteede, 1981; Giudici et al., 1999)

*Cooperia oncophora* is a nematode that has been observed to infect both cattle and sheep, but was smaller in size when recovered from a sheep host. (Smith and Archibald, 1965, Borgsteede, 1981) Borgsteede et al. observed that though most of the major genera of ruminant nematodes can be cross-infected, the size and fecundity are altered in the less natural host. Alternate grazing can be a helpful tool in the management of internal parasites, but should be used in conjunction with other anti-parasite regimens. (Rocha et al., 2008)

Nutritional supplementation is a seemingly worthy method of nematode control, as research has shown that a host animal's nutritional status is negatively correlated with intensity of parasitic burdens. (Koski and Scott, 2001; Coop and Kyriazakis, 2001) Protein-energy malnutrition (PEM) combined with deficiencies in iron, vitamin A and zinc can indirectly lead to an increase in nematode burdens. PEM can lead to decreased voluntary feed intake, lowered digestibility and absorption, and increased intestinal leakage. (Koski and Scott, 2001) An increase in protein supplementation can; 1) potentially replace the endogenous proteins that are lost due to nematode infections, 2) increase the resilience in host animals, 3) revamp the downstream effects of periparturient relaxation in immunity and 4) boost the immune response mechanisms. (Coop and Kyriazakis, 2001)

Bioactive forages ("neutraceuticals") are still under review as to their effectiveness in controlling ruminant nematodes. (Athanasiadou et al., 2001; Hoste et al., 2006; Stromberg and Archibald, 1965) There are many different bioactive plants and plant by-products that are currently being evaluated for their potential effects on internal parasites, but most research has been focused on plants that contain tannins. (Stromberg and Archibald, 1965; Coop and Kyriazakis, 2001; Athanasiadou et al., 2001; Hoste et al., 2006) The action against nematodes mediated by

condensed tannins (CTs) can be grouped into two differing methods: 1) indirectly improving the actions of the host in response to internal parasites; 2) directly binding to proteins that coat the cuticle of the nematode thus, compromising the integrity of the cuticle. (Athanasiadou et al., 2001) Though observations of lowered FEC have been observed (Coop and Kyriazakis, 2001, Athanasiadou et al., 2001, Hoste et al., 2006), supplementation with CTs can have drawbacks when administered at high levels, including a decrease in voluntary feed intake and lowered protein digestibility. (Coop and Kyriazakis, 2001, Hoste et al., 2006)

## 2. Materials and Methodology

### 2.1. Animals and Reception

#### *Cattle*

Six, 6 to 8 month old Holstein steer calves were obtained from various sale barns located in Washington County Arkansas from June to July, 2014. Fecal samples were collected upon arrival and the calves were subsequently given 15 mg/kg BW of Safe-Guard Suspension 10% (Merck Animal Health, Summit, NJ). The calves were dewormed using the Safe-Guard weekly, with the number of administrations ranging from four to seven total dewormings (Table 1a.). Starting at arrival, the calves were housed together on concrete and given mixed-grass hay, minerals and water ad libitum. Fecal samples were collected and analyzed weekly to follow strongyle egg counts using direct  $\text{MgSO}_4$  flotation of filtrate from one gram of feces per sample (Table 3a). Two of the six calves still had positive fecal strongyle egg counts at the time of inoculation (Table 3a).

Table 1a. Deworming schedule of Holstein steer calves prior to inoculation.

<i>Animal #</i>	<i>Total Treatments</i>	<i>Treatment Days Relative to Artificial Infections</i>
7	4	-20, -17, -9, -7
23C	4	-20, -17, -9, -7
30	4	-20, -17, -9, -7
42	5	-37, -31, -20, -17, -9
44	7	-44, -38, -37, -31, -20, -17, -9
45	7	-44, -38, -37, -31, -20, -17, -9

Note: All cattle were dewormed using Safe-Guard Suspension 10% (@Merck Animal Health) at a dose rate of 15 mg/kg BW.

Table 3a. Fecal egg count results for Holstein steer calves throughout the investigation.

<i>Animal</i>	<i>D-3</i>	<i>D-2</i>	<i>D7</i>	<i>D15</i>	<i>D21</i>	<i>D26</i>	<i>D29</i>	<i>D33</i>	<i>D36</i>	<i>D39</i>
7	2	2	3	0	3	13	7	22	25	35
23c	1	3	1	0	6	10	28	58	87	185
30	.	0	0	0	10	14	24	81	88	317
42	0	.	0	27	1854	1119	1416	1629	2184	1232
44	0	.	0	16	303	348	450	590	300	1004
45	0	.	0	18	368	106	200	432	423	868

Note: Fecal egg counts were quantified using direct MgSO<sub>4</sub> flotation of fecal filtrate from one gram of feces.

+ Cattle inoculated with sheep-origin nematode L<sub>3</sub>.

∞ Cattle inoculated with cattle-origin nematodes L<sub>3</sub>.

### *Sheep*

Six, 5 month old Katahdin ram lambs were obtained from one farm located in Jefferson City, Missouri in June 2014. Fecal samples were collected upon arrival and each lamb was subsequently given levamisole at 10 mg/kg via a Levasole Bolus (Merck Animal Health, Summit, NJ) combined with an oral drench and 15 mg/kg BW of Safe-Guard, at two times, as well as one deworming using 0.4 mg of moxidectin/kg BW as Cydectin Oral Sheep Drench (Boehringer Ingelheim, St. Joseph, MO) (Table 1b.). The hair sheep were housed together on dirt and given mixed-grass hay, minerals and water ad libitum.

Seven, 5 month old Suffolk X Rambouillet ewe lambs and one wether lamb were obtained from one farm located in Gaither, Arkansas in June 2014. Fecal samples were collected upon arrival

and the lambs were subsequently given levamisole at 10 mg/kg via a Levasole Bolus combined with an oral drench, at two times, 15 mg/kg BW of Safe-Guard, at two times, and 0.4 mg of moxidectin/kg BW of Cydectin Oral Sheep Drench, given once. The lambs were also given 2 grams of copper oxide wire as Copasure Bolus (Animax Limited, Bury St. Edmunds, Suffolk, England.), administered at the time of larval inoculation. (Note: Due to the late date at which they were received, two sheep were administered levamisole at 10 mg/kg via Levasole Bolus combined with an oral drench, at two times, 15 mg/kg BW of Safe-Guard, at two times, and 2 grams of copper oxide wire as Copasure Bolus at the time of inoculation; no Cydectin Oral Sheep Drench was administered due to the long retention time of moxidectin in the adipose tissues (Table 1c).) The wool sheep were housed together on dirt and given mixed-grass hay and minerals ad libitum.

Table 1b. Deworming schedule of Katahdin ram lambs prior to inoculation.

<i>Animal #</i>	<i>Total Treatments</i>	<i>Treatment Days Relative to Artificial Infections</i>		
		<i>Safe-Guard</i>	<i>Levasole</i>	<i>Cydectin</i>
10	5	-66, -51	-66, -51	-38
11	5	-66, -51	-66, -51	-38
12	5	-66, -51	-66, -51	-38
13	5	-66, -51	-66, -51	-38
14	5	-66, -51	-66, -51	-38
15	5	-66, -51	-66, -51	-38

Note: Dosage rates administered: 15 mg/kg BW of Safe-Guard Suspension 10% (Merck Animal Health), 10 mg/kg of Levasole Bolus (Merck Animal Health) as an oral drench and 0.4 mg/kg BW of Cydectin Oral Drench (Boehringer Ingelheim).

Table 1c. Deworming schedule of Suffolk X Rambouillet X ewe and wether lambs prior to inoculation.

Animal #	Total Treatments	Treatment Days Relative to Artificial Infections			
		Safe-Guard	Levasole	Cyductin	Copasure
18	5	-16, -7	-16, -7	N/A	0
19	5	-16, -7	-16, -7	N/A	0
20	7	-53, -51	-66, -51, -43	-38	0
21	7	-53, -51	-66, -51, -43	-38	0
22	7	-53, -51	-66, -51, -43	-38	0
23S	7	-53, -51	-66, -51, -43	-38	0
24	7	-53, -51	-66, -51, -43	-38	0
25	7	-53, -51	-66, -51, -43	-38	0

Note: Dosage rates administered: 15 mg/kg BW of Safe-Guard Suspension 10% (Merck Animal Health), 10 mg/kg of Levasole Bolus (@Merck Animal Health) as an oral drench, 0.4 mg/kg BW of Cyductin Oral Drench (Boehringer Ingelheim) and 2 grams of Copasure Bolus (Animax Limited).

Fecal samples were collected and analyzed periodically from all lambs to follow the strongyle egg counts using direct MgSO<sub>4</sub> flotation of filtrate from one gram of feces from each sample or McMaster's fecal egg counting technique (Table 3b/Table 3c). All eight wool sheep held positive fecal strongyle egg counts at the time of inoculation (Table 3b).

Table 3b. Fecal egg count results for Katahdin ram lambs throughout the investigation.

Animal	D-3	D-2	D7	D15	D21	D26	D29	D33	D36	D40
10+	0	.	0	0	405	345	768	2400	.	7650
11+	0	.	0	0	356	1014	3050	5900	5750	5150
12+	0	.	0	0	293	717	2700	4950	4900	5050
13∞	.	0	0	0	3105	1818	7550	16200	11950	12200
14∞	.	0	0	35	369	243	714	2600	2500	1800
15∞	0	.	0	5	144	350	792	4050	3100	3500

Note: Fecal egg counts were quantified using direct MgSO<sub>4</sub> flotation of fecal filtrate from one gram of feces (EPG<500) or modified McMaster fecal egg counting technique using fecal filtrate from one gram of feces (EPG>500).

+Lambs inoculated with sheep-origin nematode L<sub>3</sub>.

∞Lambs inoculated with cattle-origin nematodes L<sub>3</sub>.



Table 3c. Fecal egg count results for Suffolk X Rambouillet lambs throughout the investigation.

Animal	D-3	D-2	D7	D15	D21	D26	D29	D33	D36	D40
18 $\infty$	508	735	0	5	89	236	591	1550	3050	4150
19+	645	504	209	613	2316	.	.	.	.	.
20+	798	693	82	169	2056	12100	24250	25850	14150	13500
21-	.	328	267	276	272	220	435	279	900	550
22 $\infty$	204	68	126	375	924	2994	3550	4200	24500	3700
23s+	724	168	60	16	255	1575	2350	2100	2900	2850
24-	300	336	74	67	204	83	300	173	450	250
25 $\infty$	301	192	36	171	749	1272	2150	3000	5150	3600

Note: Fecal egg counts were quantified using direct MgSO<sub>4</sub> flotation of fecal filtrate from one gram of feces (EPG<500) or modified McMaster fecal egg counting technique using fecal filtrate from one gram of feces (EPG>500).

+Lambs inoculated with sheep-origin nematode L<sub>3</sub>.

$\infty$ Lambs inoculated with cattle-origin nematodes L<sub>3</sub>.

## 2.2. Parasitological Procedures

### 2.2.1. Inoculations

Animals were inoculated with infective strongyle larvae that were cultured from cattle and sheep feces collected from Northwest Arkansas/Southeast Oklahoma/Mid-Missouri. Larval identification and quantification was conducted by Dr. Yazwinski.

Three cattle, three hair sheep and three wool sheep were each inoculated with cattle-origin *Cooperia oncophora* (85,555), *Cooperia punctata* (53,888), *Haemonchus placei* (10,666), *Ostertagia ostertagi* (9,000) and *Oesophagostomum radiatum* (2,333) on August 7, 2014.

In addition, three cattle, three hair sheep and three wool sheep were each inoculated with sheep-origin *Trichostrongylus colubriformis* (78,222), *Haemonchus contortus* (68,555) and *Oesophagostomum venulosum* (1,111) on August 7, 2014.

Two wool breed sheep were not inoculated with any L<sub>3</sub> and were kept as control animals for comparison to the other wool breed sheep that were infected.

### 2.2.2. Coprology

Fecal samples were collected, processed and analyzed from all animals before and throughout the investigation. Strongyle egg counts were obtained and recorded using either direct centrifugation fecal flotations, a procedure that uses high specific gravity to concentrate helminth eggs, or modified McMaster fecal egg counting techniques, which quantifies large numbers of nematode eggs in the absence of centrifugation. Direct fecal flotations were used if the feces source was cattle or the previous eggs per gram (EPG) was under 500 EPG; the McMaster's technique was used in the previous sheep fecal sample help over 500 EPG. Coprocultures were conducted using a minimum of twenty grams of feces homogenized with a minimum of five grams of vermiculite. The coprocultures were allowed to sit in a warm room for thirteen to sixteen days then filled with water and inverted to allow for the collection of the larvae. Larvae were placed into individual Pyrex centrifuge tubes, killed with 10% Formalin and stretched by transient boil. The samples sat overnight to allow for settling of the larvae. The excess fluid was pipetted out of the tube and the remainder was homogenized. A sample from each tube was pipetted onto a microscope slide for quantification and identification (Table 4) (Van Wyk et al., 2013).

Table 4. Average percentage of infective larvae quantified from treatment group coprocultures.

Treatment	<i>H. placei</i>	<i>H. contortus</i>	<i>Ostertagia spp</i>	<i>C. oncophora</i>	<i>C. punctata</i>	<i>Oesophagostomum spp</i>	<i>Trichostrongylus spp</i>
1	41.2	32.0	11.4	4.8	10.4	0	0.2
2	20.3	0	20.0	15.7	39.2	4.8	0
3	0	59.5	0	0	0	0	40.5
4	25.6	1.1	22.7	11.9	38.2	0.3	0.2
5	10.5	59.9	4.8	5.1	19.0	0.3	0.2
6	0	87.5	0	0	0	0.3	12.2
7	0	97.3	0	0	0	0	2.7

Note: Infective larvae were obtained and processed 12 to 15 days post coprocultures.

Treatment groups: 1=Cattle infected with sheep nematodes, 2=Cattle infected with cattle nematodes, 3=Katahdin sheep infected with sheep nematodes, 4=Katahdin sheep infected with cattle nematodes, 5=Wool sheep infected with cattle nematodes, 6=Wool sheep infected with sheep nematodes, 7=Wool sheep not infected with nematodes

### 2.2.3. Aliquot and Digest Preparation

The animals were killed by host species on consecutive days by captive bolt and exsanguination at the University of Arkansas Abattoir. The intestinal contents were removed immediately following death and were processed for parasite collection. The abomasums, small intestines and large intestines were ligated and separated for content collections. The abomasum was bound by ligatures at the omaso-abomasal orifice and at the pyloric sphincter; the small intestine bound by ligatures at the pyloric sphincter and at the ileocecal valve; the large intestine and cecum bound by ligatures at the ileocecal valve and at the descending colon. The individual organs were opened lengthwise and their contents emptied into a container, brought up to 3 liters using water, and a 5% (150 mL) aliquot removed and preserved with 10% Formalin. The organs were allowed to soak for four hours (small intestine and large intestine/cecum) or overnight (abomasum). After soaking, the organs were removed, washed and the total residue was preserved with 10% Formalin.

### 2.2.4. Parasite Isolation and Quantification Procedures

#### 1. The abomasal 5% content aliquot:

For each sample, the content aliquot was washed over a #60 (250  $\mu\text{m}$ ) sieve and all residue and filtrate were collected separately. The residue was collected via backwash and made up to 1 L (Residue 1). The filtrate was washed over a #200 (74  $\mu\text{m}$ ) sieve and the residue was backwashed, collected and made up to 1 L (Residue 2).

With homogenization, Residue 1 was stereoscopically viewed in 10-20 mL subsamples until the total was viewed. The same exact percentage analyzed was applied to Residue 2.

(Note: If the contents were too concentrated, then a 1% aliquot was processed.)

## 2. The abomasal digest:

The collected digest fluid was made up to 4 liters, and a 5% subsample (200 mL) was removed during homogenization and washed over a #400 (37  $\mu\text{m}$ ) sieve. The residue was collected via backwash and made up to 1000 mL. During homogenization, 10 to 20 mL subsamples were removed and viewed stereoscopically. This was continued until the total residue was viewed. All nematodes were identified, quantified and recorded.

## 3. The small intestine 5% content aliquot:

The same procedure that was used for the abomasal 5% content aliquot was used for the small intestine 5% content aliquot except that Residue 2 was washed over a #120 (125  $\mu\text{m}$ ) sieve.

(Note: If the contents were too concentrated, then a 1% aliquot was processed.)

## 4. The small intestine digest:

The same procedure that was used for the abomasal digest detailed above was used to identify, quantify and record the nematodes found in the small intestine digest.

#### 5. The large intestine/cecum 5% content aliquot:

The content aliquot for each sample was washed over a #35 (500  $\mu\text{m}$ ) sieve and the residue was viewed stereoscopically in total (100%). All nematodes recovered were identified, quantified and recorded.

#### 6. The large intestine/cecum digest:

The same procedure that was used for the abomasal digest detailed above was used to identify, quantify and record the nematodes found in the large intestine/cecum digest except that a 120  $\mu\text{m}$  sieve is used. The entire (100%) digest residue was viewed stereoscopically.

#### 2.2.5. Mathematics

Arithmetic means were calculated for the fecal egg counts using Statistical Analysis Software (SAS).

### 3. Results

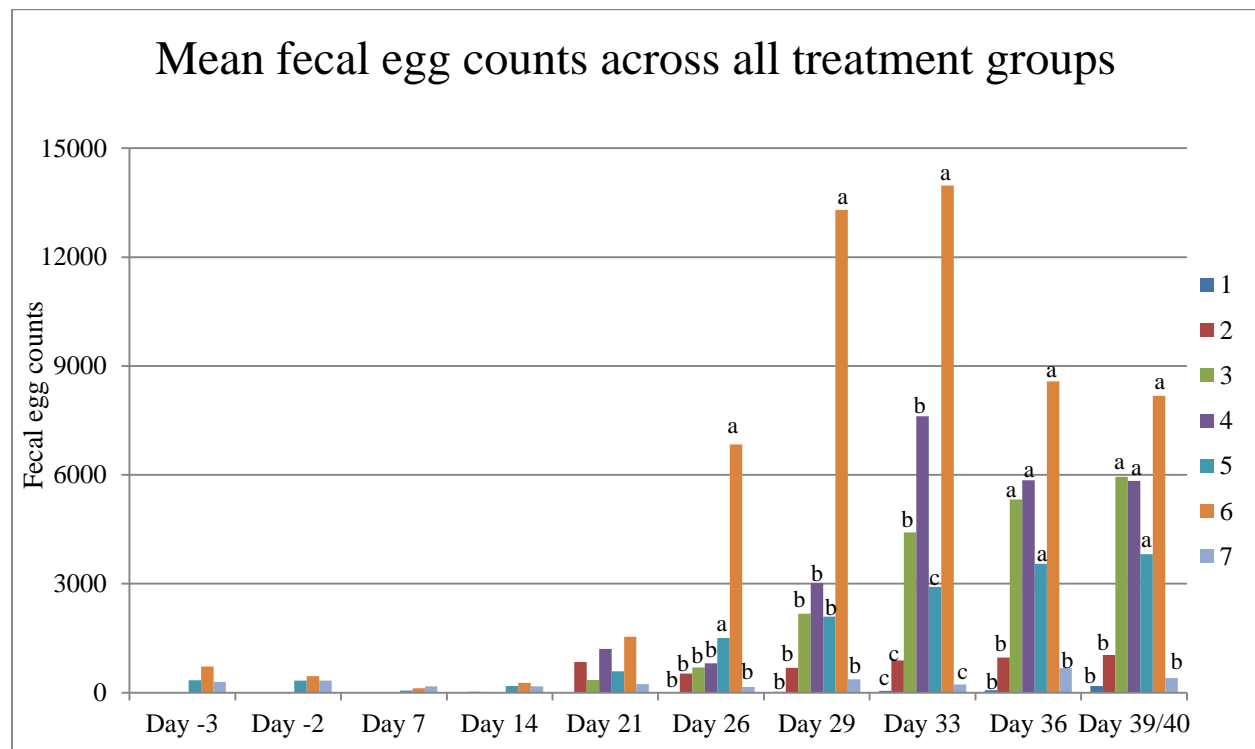
#### 3.1. Coprology

##### Fecal egg counts and larval identification

The mean fecal egg counts were calculated for each treatment group on a day-basis (Figure 1). There were no significant differences for the first five fecal collections. On days 26 and 29, treatment group 5 and 6, and treatment 6, respectively, was found to be significantly different than the remaining treatment groups. On day 33, treatment groups 1, 2 and 7 are significantly lower than treatment groups 3 and 4; treatment group 6 is significantly higher than all other

treatments. On days 36 and necropsy (day 39/40), the mean FEC for treatment groups 3, 4, 5 and 6 were significantly higher than the remaining treatment groups.

Figure 1. The arithmetic mean for fecal egg count results across all treatment groups throughout the course of the investigation.



Note: Fecal egg counts were quantified using direct  $\text{MgSO}_4$  flotation (EPG <500) or modified McMaster's flotation technique (EPG >500) of fecal filtrate from one gram of feces.

Treatment groups: 1=Cattle infected with sheep nematodes, 2=Cattle infected with cattle nematodes, 3=Katahdin sheep infected with sheep nematodes, 4=Katahdin sheep infected with cattle nematodes, 5=Wool sheep infected with cattle nematodes, 6=Wool sheep infected with sheep nematodes, 7=Wool sheep not infected with nematodes.

a, b, c, d, e Day specific means with unlike superscripts are different ( $p < 0.05$ ) within study day.

The total arithmetic mean fecal egg counts for each treatment group is show in Table 2.

Treatment group 6 held the highest FEC, while treatment group 1 held the lowest.

Table 2. Arithmetic mean ( $\pm$  SE) for fecal egg count results for all treatment groups throughout the investigation.

Treatment	<i>Fecal Egg Counts</i>	
	$\bar{x}$	( $\pm$ ) SEM
1	30	12.19
2	496	114.56
3	1172	445.03
4	2434	759.81
5	1573	292.36
6	4441	1492.36
7	303	43.75

Note: Fecal egg counts were quantified using direct MgSO<sub>4</sub> flotation of fecal filtrate from one gram of feces.

Treatment groups: 1=Cattle infected with sheep nematodes, 2=Cattle infected with cattle nematodes, 3=Katahdin sheep infected with sheep nematodes, 4=Katahdin sheep infected with cattle nematodes, 5=Wool sheep infected with cattle nematodes, 6=Wool sheep infected with sheep nematodes, 7=Wool sheep not infected with nematodes.

### 3.2. Adult quantification

The total number of nematodes was calculated for each treatment group. More *H. placei* were found in the treatment groups that were given cattle-source inoculations, although populations of these nematodes were also found in the sheep-source inoculates, as well as the control group in small numbers (Figure 2a). The greatest population of *H. contortus* adults was obtained from the wool sheep that were administered sheep-source L<sub>3</sub>, though all sheep-source treatment groups help greater populations than the cattle-source treatment groups. *H. contortus* was found in the control animals in small amounts (Figure 2b). *Ostertagia ostertagi* was found in the cattle treatment groups (both inoculate sources), with the highest being from the cattle-source inoculation. *O. ostertagi* was isolated from sheep-source wool treatment group, as well as, cattle-source hair and wool treatment groups, but the total numbers were below 100 nematodes (Figure 2c). *Oesophagostomum radiatum* was obtained from only two treatment groups: cattle-source cattle and hair treatment groups (Figure 2d). The highest population (by 790 nematodes) was the cattle treatment group. *Oesophagostomum venulosum* was found in all treatment groups, save

cattle given cattle-source inoculates. The greatest numbers were observed in sheep-source hair sheep. (Figure 2e). *C. punctata* adults were isolated from all three cattle-source treatment groups, with the highest being cattle and the lowest being wool sheep. Small numbers of *C. punctata* were quantified from the sheep-source cattle treatment group (Figure 2f). Similar proportions were found to be true in *C. oncophora*, as well (Figure 2g). *T. colubriformis* was found in the three sheep-source treatment groups, as well as both cattle-source and control wool sheep treatment groups (2h).

Figure 2a. Adult populations of *Haemonchus contortus* across all treatment groups.

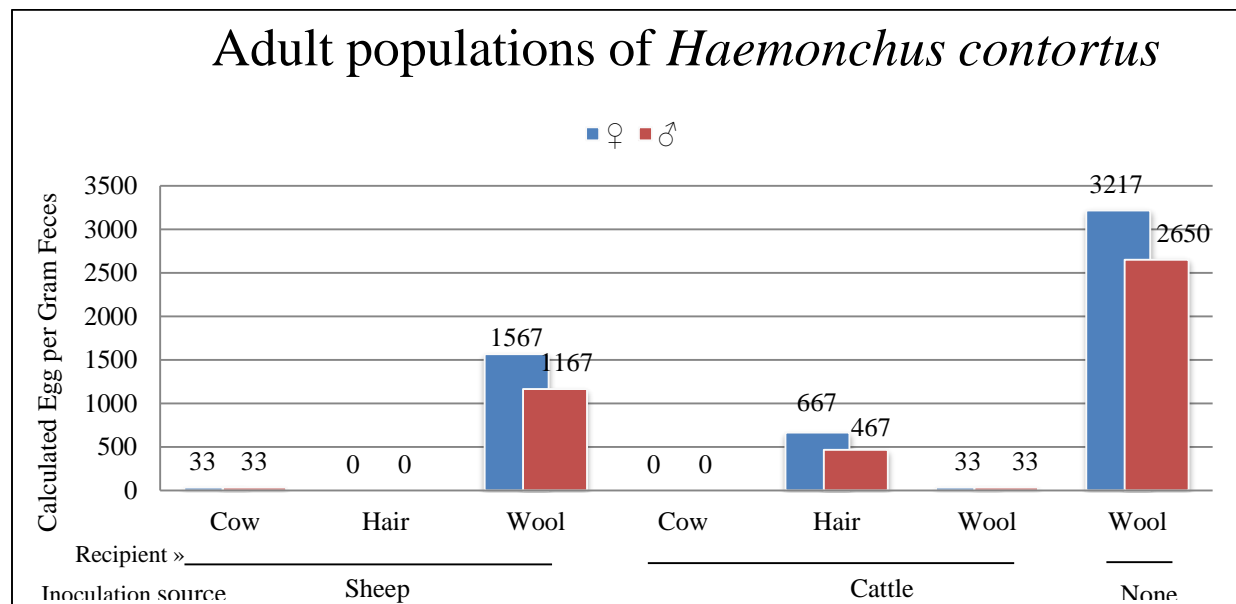




Figure 2b. Adult nematode populations of *Haemonchus contortus* across all treatment groups.

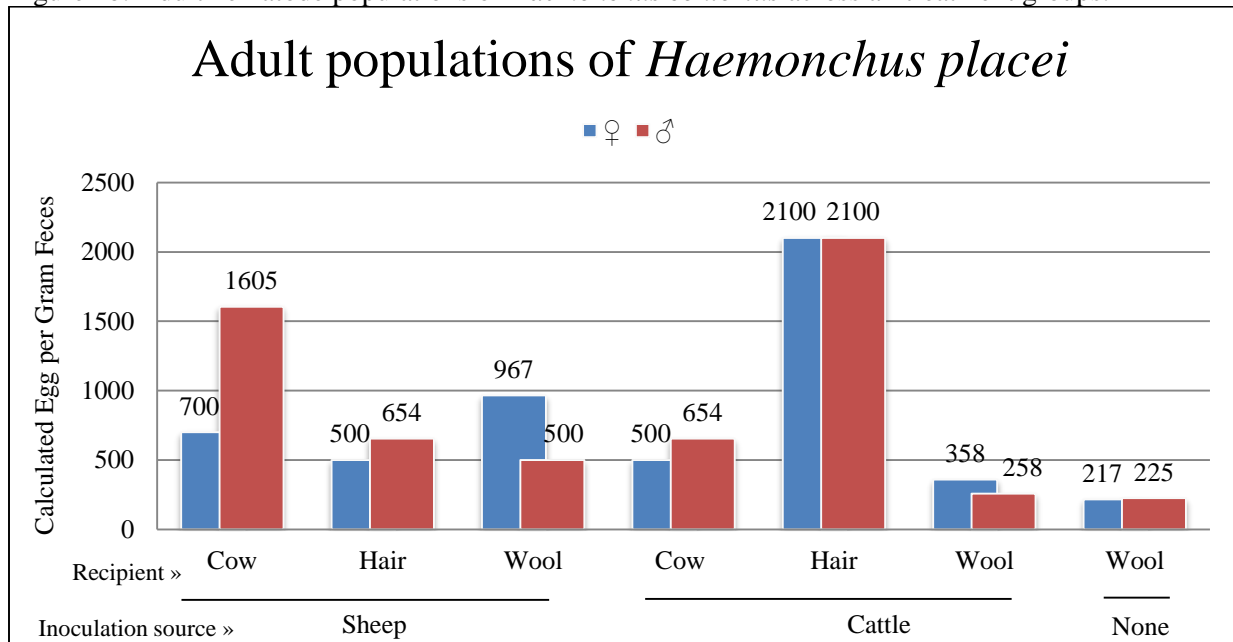


Figure 2c. Adult nematode populations of *Ostertagia ostertagi* across all treatment groups.

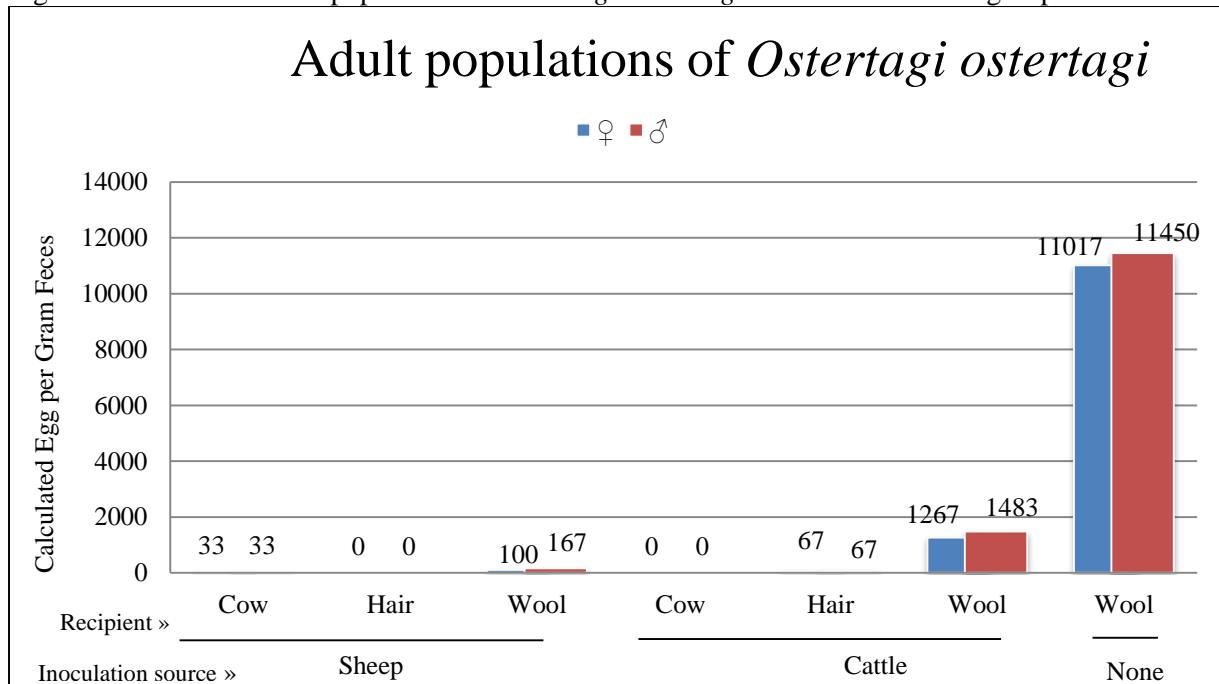


Figure 2d. Adult nematode populations of *Oesophagostomum radiatum* across all treatment groups.

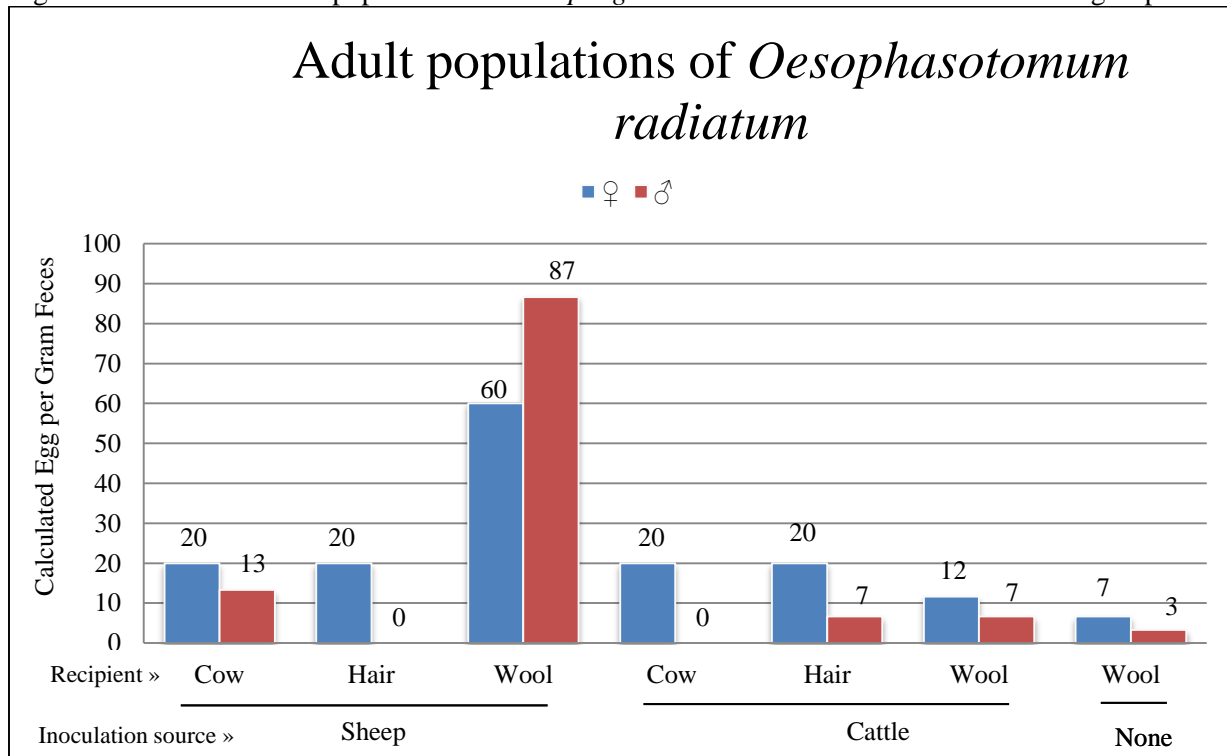


Figure 2e. Adult nematode populations of *Oesophagostomum venulosum* across all treatment groups.

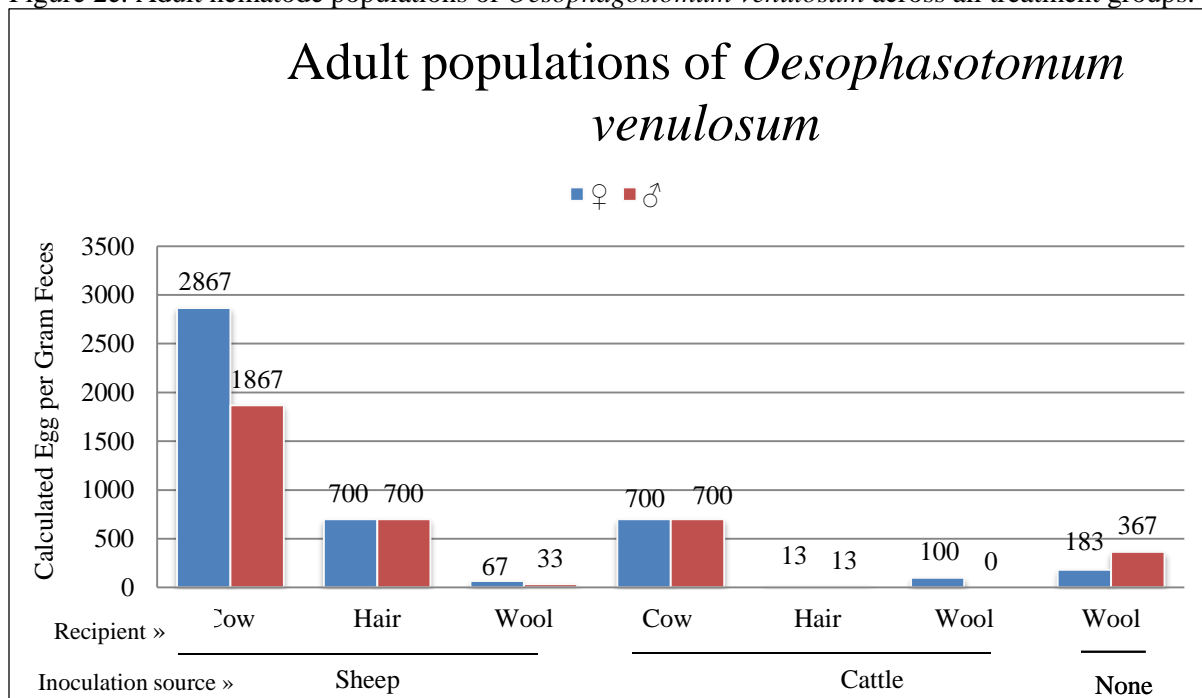


Figure 2f. Adult nematode populations of *Cooperia punctata* across all treatment groups.

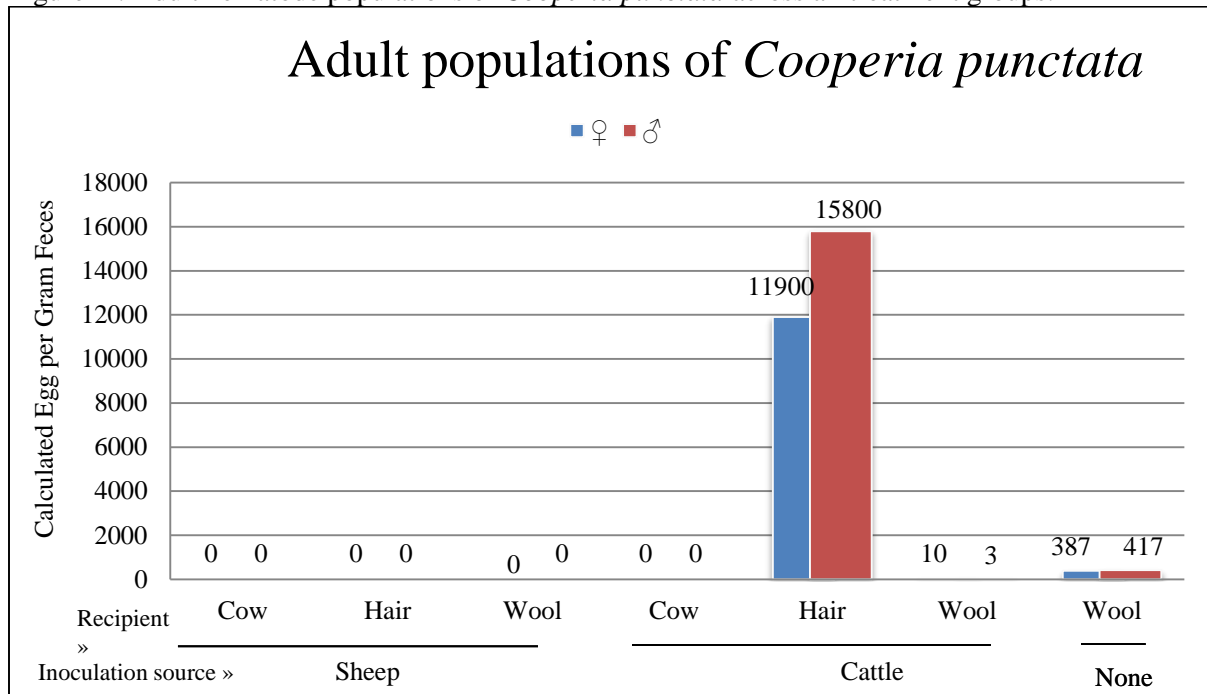


Figure 2g. Adult nematode populations of *Cooperia oncophora* across all treatment groups.

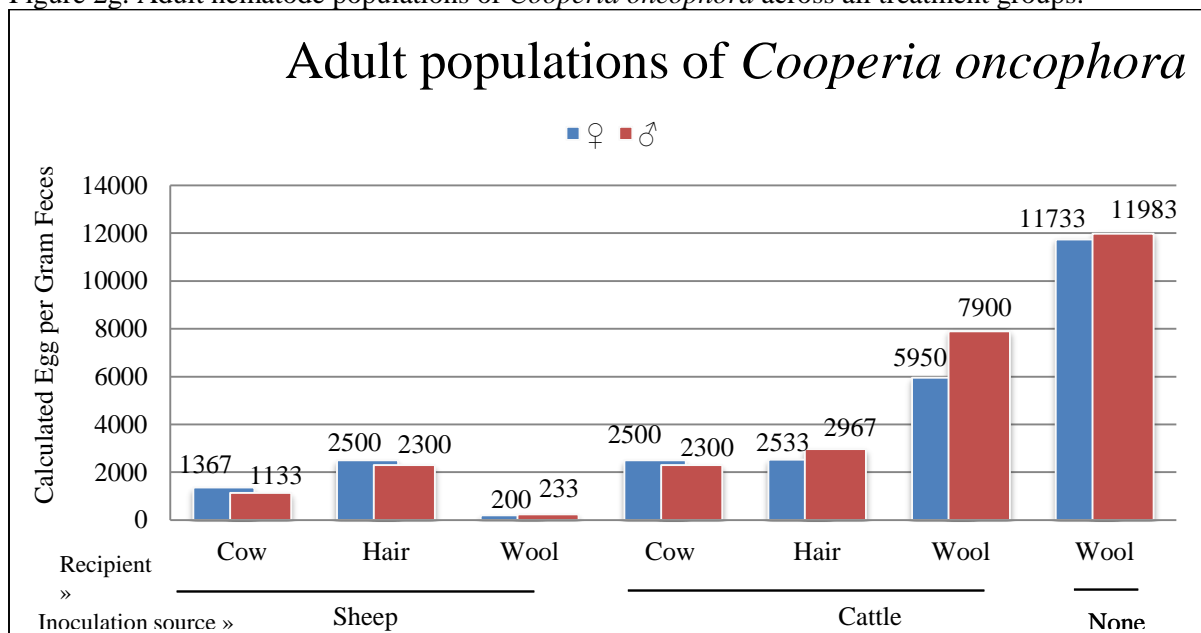
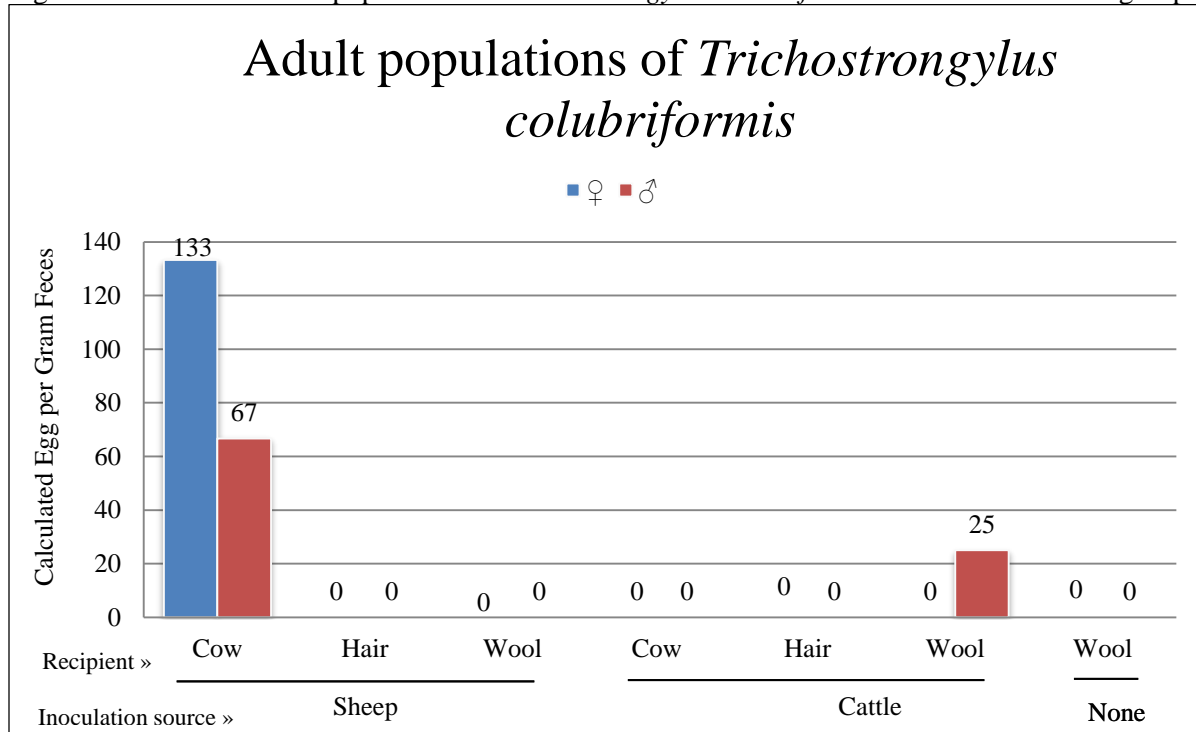


Figure 2h. Adult nematode populations of *Trichostrongylus colubriformis* across all treatment groups.



#### 4.1. Discussion

The mean fecal egg counts (FEC) on a day-basis show increasing statistical differences ( $p > 0.05$ ) at the end of the investigation starting with treatment 6 (wool sheep inoculated with sheep-source nematodes) showing a significant difference on days 26 and 29; this groups also held underlying FEC on the day of inoculation, so the elevated egg counts are to be expected.

On day 33, treatment groups 3 (hair sheep inoculated with sheep-source nematodes), 4 (hair sheep inoculated with cattle-source nematodes) and 6 shed much higher EPG in comparison with treatment groups 1 (cattle inoculated with sheep-source nematodes), 2 (cattle inoculated with cattle-source nematodes) and 7 (wool sheep control animals). Treatment group 4 having comparable FEC is unexpected.

Days 36 and 39/40 (necropsy) show a similar spread in FEC, with treatment groups 3, 4, 5 (wool sheep inoculated with cattle-source nematodes) and 6 having significantly higher EPG than treatment groups 1, 2 and 7. The elevated FEC of the sheep carrying cattle-source nematodes is unexpected, especially when the FEC rival the numbers of eggs being passed by the sheep carrying sheep-source nematodes.

The low fecal egg output by group 1 shows that using cattle as models for the sheep-source nematodes can keep the fecundity of the nematodes low, but this must be taken in context of reality. The elevated FEC of the sheep carrying cattle-source nematodes should make one hesitate before implementing cattle and sheep co-species grazing into standard operations.

The total arithmetic mean FECs for the entire study (Table 2) were highest amongst treatment group 6 and 4, respectively. Treatment 6 held high adult populations of both species of *Haemonchus* spp. (Figure 2a and 2b). Treatment 4 held high adult populations of both species of *Haemonchus* spp. (Figure 2a and 2b) and *Cooperia* spp. (Figure 2f and 2g). Treatment group 5 held the third highest FEC for the entire study (Table 2). This group had high adult populations of *H. placei* (Figure 2b) and *C. oncophora* (Figure 2g).

Both cattle treatment groups (1 and 2) held relatively low FEC throughout the investigation, with the sheep-source (treatment 1) inoculates holding the lower of the two (Table 2). The cattle that were inoculated with the sheep-source L<sub>3</sub> had high adult populations of the sheep-specific nematodes *O. venulosum* (Figure 2d) and *T. colubriformis* (Figure 2h), but also the cattle-specific *H. placei* (Figure 2b). The cattle that were inoculated with cattle-source L<sub>3</sub> had elevated adult populations of *H. placei* and *C. punctata* (Figure 2f).

The wool control group held elevated adult populations of *H. contortus* (Figure 2a), *O. ostertagi* (Figure 2c) and *C. oncophora* (Figure 2g); *H. placei* most likely accounts for the majority of fecal egg output.

Overall, the cattle that were given sheep-source nematodes (treatment 1) did hold low FEC throughout the entire investigation, which could be a promising find for sheep producers who are having trouble with multidrug-resistant nematodes on their operations. However, a pause should be taken; the sheep treatment groups that were given cattle-source nematodes (treatments 4 and 5) had comparable FECs to the sheep treatment groups that were given sheep-source nematodes (treatments 3 and 6), indicating that sheep make a suitable host for both cattle and sheep nematodes.

#### 4.2. Factors that influenced this project

Four of the cattle that were originally obtained for this study had to be removed due to sickness and replaced by cattle that could not be completely rid of their nematode burdens before the initiation of the study. Two of these cattle held very low FECs, and were given the sheep-source L<sub>3</sub> inoculations in order to attempt to “clean” up the data. One steer was a few months older and bigger than the remaining steers, which could be a factor in his lowered FEC when compared to the remaining cattle (Table 3a). The replacement steers were given fewer total deworming treatments, which could also account for the decreased overall FECs throughout the course of the investigation. Along these same parameters, the Suffolk X Rambouillet wool sheep were obtained from a farm which unknowingly contained resistant nematodes. This entire group was given three different classes of anthelmintic, as well as a novel treatment, yet all held positive

FECs on Day 0 of the investigation (Table 1c). In treatment group 6, an animal died of anemia on day 21, so the mean shown by day is seemingly very large (Figure 1).

Also, very small numbers of two species of nematodes were found that the animals were not inoculated with: *Chabertia ovina* and *Teladorsagia circumcincta*. *C. ovina* was easily identified by their distinguishing head. *T. circumcincta* were distinguished from *O. ostertagi* by measuring male spicule length. The discovery of these two parasites is further evidence that the animals were not sufficiently ridged of their parasite burdens prior to the start of the investigation.

Identification of nematode L<sub>3</sub> is a skill that one must master over a long period of constant practice, thus creating flaws in the obtained data; this led to the decision to exclude this data from the results. The identification of adult nematodes, though less difficult and with less room for error in comparison with L<sub>3</sub> identification, did not come without its flaws. The adult *Oesophagostomum* spp spicules were too obscure to measure, so they were identified by their heads. *O. venulosum* possesses an external leaf crown that *O. radiatum* does not. The hair and wool sheep that were given cattle-source L<sub>3</sub> had adult nematodes that looked more like *O. venulosum* than *O. radiatum*, but this likeness was subjective, at best (Figure 3h.). The adult male *Haemonchus* spp were identified by the length of their spicules, but there was some overlap in measurements, so these identifications are estimations.

Adult specimens collected from the abomasum contents of the animals in this investigation have been preserved in ethanol, rather than formaldehyde, and will be sent to Dr. John Gilliard's laboratory in Calgary, Canada for DNA identification; unfortunately, only the abomasum contents were preserved in ethanol, so future proper identification can only occur for *Haemonchus* spp and *Ostertagi* spp.

## 5. Implications

Based on this investigation's results, the recommendation to co-species graze using cattle and sheep as a means of parasite control is not one that should be, in good-coconscious, be doled out to producers. The sheep-source nematodes shed much lower numbers, and fewer adults were collected at necropsy, when using cattle models. However, the cattle-source nematodes shed rivalling numbers of eggs when comparing cattle and sheep models; this leads the investigator to surmise that all ruminant nematodes are rather successful when using sheep models.

Also, the content of this investigation could, and should, be expanded upon using differing evaluation methods at each step of analyzation. The issues that arise when using naked or microscopic visualization can obscure the data when encountering things like overlapping measurements across two different genus's of the same species, identification of nematode L<sub>3</sub>, assessing the morphological variations among closely related nematode species, etc. Comparing the traditional visualization parasitological procedures to analyzation at the DNA level should be conducted to assess the overall value of the current procedures.

Being able to possess truly naïve animals is quite necessary in order to gain baseline data. If this investigation had been fortunate enough to contain a uniformity of naivety, perhaps the results would be altered and, thus, more appropriate for founded interpretation.



## 6. Citations

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## 7. Appendix

Figure 3a. Adult male *Haemonchus placei* (100X magnification).



Figure 3b. Adult male *Haemonchus contortus* (100X magnification).





Figure 3c. Adult male *Ostertagia ostertagi* (100X magnification).



Figure 3d. Adult male *Cooperia punctata* (100X magnification).



Figure 3e. Adult male *Cooperia oncophora* (100X magnification).



Figure 3f. Adult male *Oesophagostomum radiatum* (100X magnification).



Figure 3g. Adult male *Oesophagostomum venulosum* (100X magnification).



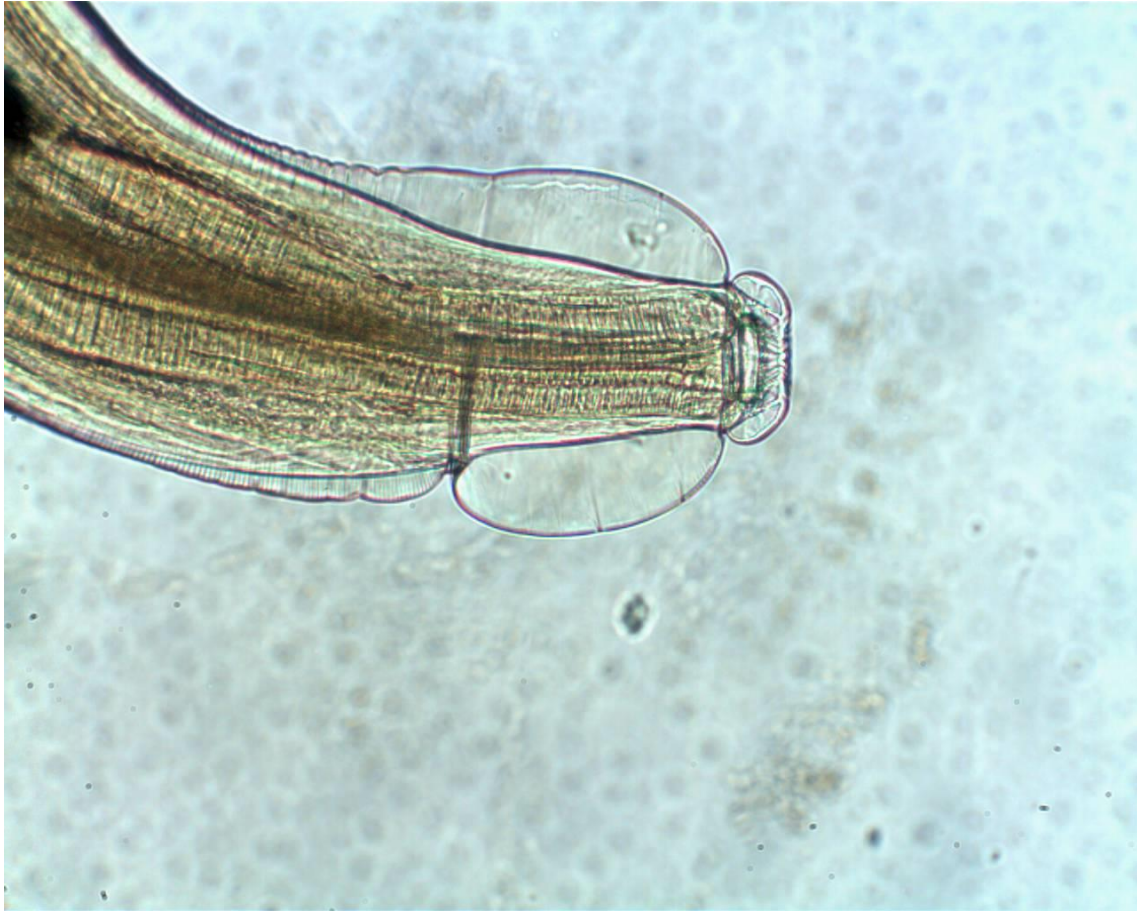


Figure 3h. Obscure adult male *Oesophagostomum venulosum* (100X magnification).

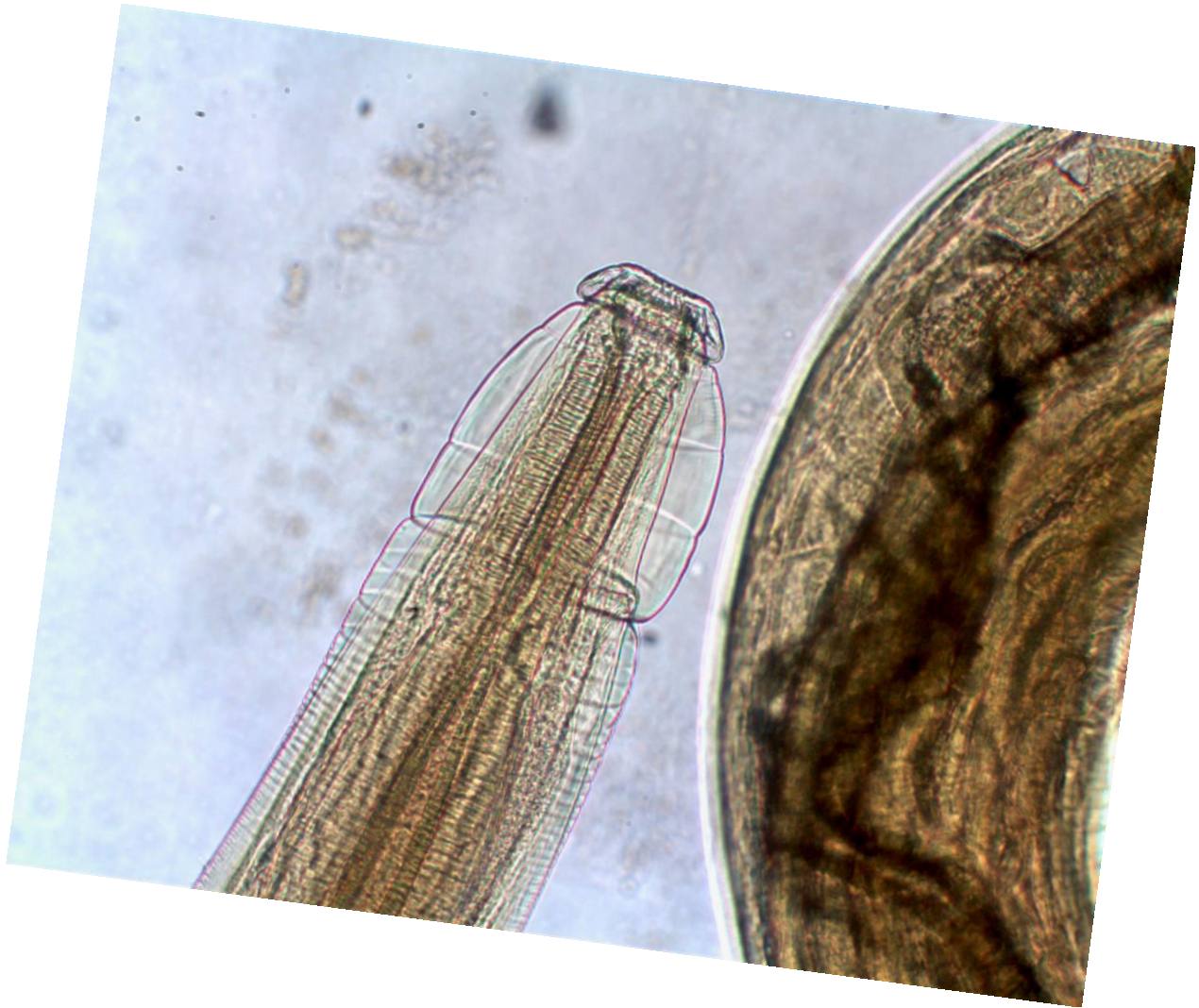
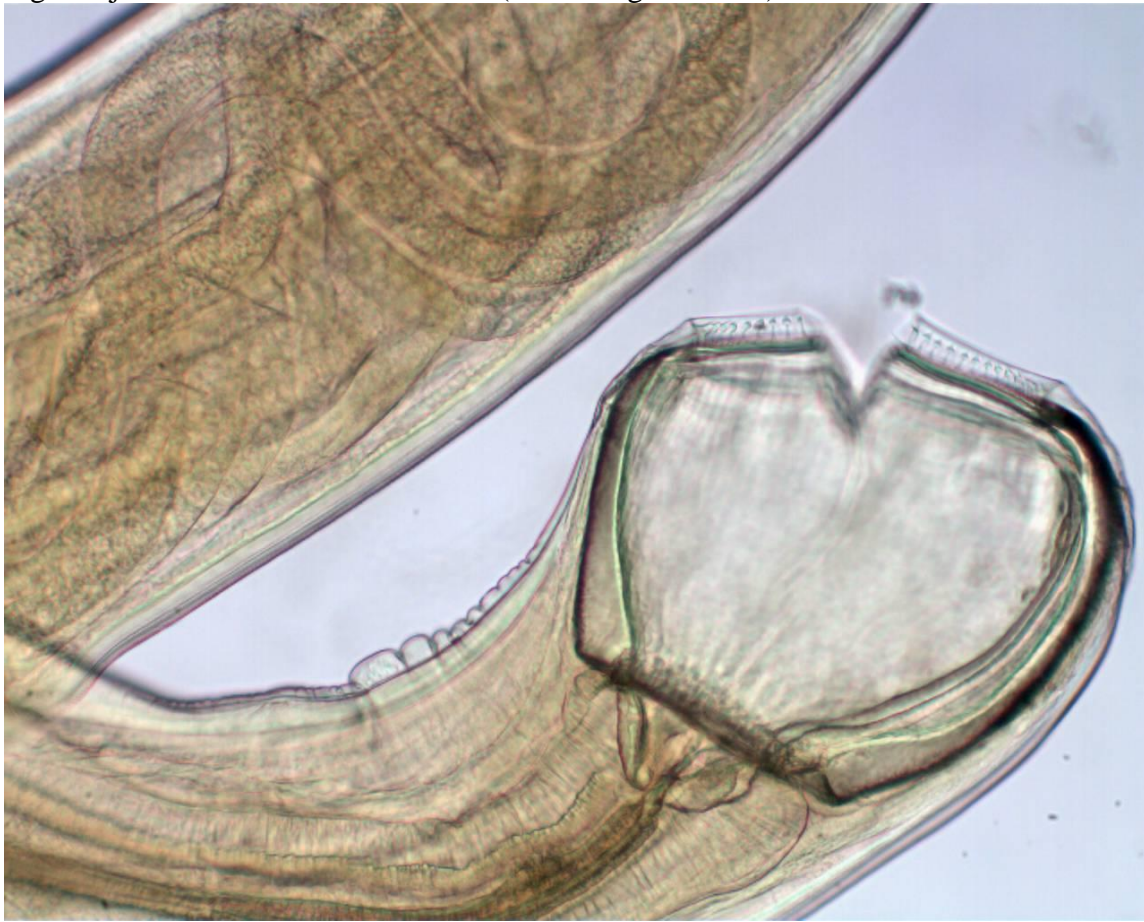


Figure 3i. Adult male *Trichostrongylus colubriformis* (100X magnification).





Figure 3j. Adult male *Chabertia ovina* (100X magnification).







MEMORANDUM

TO: Dr. T A Yazwinski

FROM: Craig N. Coon, Chairman  
Institutional Animal Care and Use Committee

DATE: June 12, 2014

SUBJECT: IACUC APPROVAL  
Expiration date: June 11, 2017

The Institutional Animal Care and Use Committee (IACUC) has APPROVED your protocol 14053: "Investigations into the cross- infectivity of nematode parasites of sheep and cattle, with assessment of co-grazing as a means of parasite control " .

In granting its approval, the IACUC has approved only the information provided. Should there be any further changes to the protocol during the research, please notify the IACUC in writing(via the Modification form) prior to initiating the changes. If the study period is expected to extend beyond June 11, 2017 you must submit a new protocol. By policy the IACUC cannot approve a study for more than 3 years at a time.

The IACUC appreciates your cooperation in complying with University and Federal guidelines involving animal subjects.

CNC/aem

cc: Animal Welfare Veterinarian